la saisit et alla se placer en position d'accouplement (fig. 52 et pl. 1, fig. 2), en se cramponnant à l'aide de ses $Mx_2$ sur la partie postérieure de sa partenaire; ainsi installé, le mâle se mit à palper le dernier segment du corps de la femelle avec ses appendices buccaux, puis il recourba son abdomen jusqu'à le faire passer entre ses $Mx_2$, répéta rythmiquement ce mouvement, comme s'il frottait l'extrémité de son abdomen entre ses deux $Mx_2$; après quelques instants, il se redressa, puis recommença son manège; après une dizaine de minutes, les spermatophores furent éjaculés et mis en place, le mâle quitta la femelle, regagna la peau du Poisson qu'il recommença à ronger. Observée au microcope une fois fixée, cette femelle avait bien été fécondée.

J'ai pu observer une fois un mâle isolé de toute femelle et qui frottait l'extrémité de son abdomen entre ses $Mx_2$. Comme il n'y a pas de copulation proprement dite entre mâle et femelle, mais seulement dépôt des deux spermatophores sur le dernier segment de celle-ci, il ne serait pas impossible qu'un mâle qui n'a pas trouvé de femelle provoquât l'éjaculation de ses spermatophores.

Lors de la fécondation normale, le mâle évacue donc entre ses $Mx_2$ ses spermatophores et les colle sur les orifices des deux vagins, auxquels ils sont toujours exactement abouchés; le contenu des spermatophores se déverse dans les voies sexuelles femelles et les spermatozoïdes vont s'emmagasiner à l'intérieur du réceptacle séminal impair où ils attendent la maturité des œufs; les coques vides des deux spermatophores adhèrent aux orifices vaginaux de la femelle durant toute sa vie et sur les femelles adultes de *S. mattheyi* capturées dans la nature, on ne trouve jamais plus de deux de ces coques; c'est sur cette constatation que nous basons notre hypothèse que les femelles ne s'accouplent qu'une fois en leur vie. Par contre, N. Fasten qui a étudié la biologie de *Salmincola edwardsii*, a observé sur les femelles de ce parasite jusqu'à six coques de spermatophores adhérant au dernier segment et il en a conclu que les femelles s'accouplaient en tout cas trois fois. Dans notre cas, l'accouplement a lieu au stade VI: les mâles meurent de un à trois jours après, tandis que quelques jours plus tard, les femelles se métamorphosent une sixième fois; c'est au cours de ce septième stade qu'elles acquerront leur complet développement. Si nous considérons les différences qui se présentent entre les femelles du stade VI et celles du stade VII et si nous insistons sur le fait qu'au moment de la

fécondation l'appareil sexuel femelle est encore en grande partie à l'état d'ébauche, nous pouvons dire que le stade VI, définitif pour le mâle, est encore un stade larvaire en ce qui concerne la femelle.

* * *

Il ne nous a malheureusement pas été possible d'assister à la dernière mue de la femelle; d'après quelques constatations préliminaires, interrompues par les événements de cette fin d'année 1939, il nous a seulement été possible d'établir que cette mue se produit entre le sixième et le vingt-cinquième jour après la fécondation. La transformation la plus frappante que nous présente le stade VII (pl. 1, fig. 1), est celle de l'organe maxillaire de fixation: les $Mx_2$ s'allongent, se développent, et le pédoncule qui les termine se transforme en un grand bouton de fixation. F. Zandt décrit cette métamorphose de l'appareil fixateur chez l'*Achtheres pseudobasanistes* où la formation du disque commence après la sixième mue: « findet eine Massenausscheidung von Sekret in der ganzen Länge des Haftfadens durch Poren statt; dieses erstarrt in dem umgebenden Wasser rasch. Die lotrechte Richtung des Sekretflusses zu der Richtung des Haftfadens lässt sich leicht an der erstarrten Masse feststellen. »

Parallèlement, nous pourrions conjecturer des faits semblables chez *Salmincola mattheyi:* la sécrétion des masses glandulaires frontales sortirait à l'extrémité des $Mx_2$ et de là serait injectée sous l'épiderme du Poisson. Il nous semble peu probable que le pédoncule primitif puisse servir de canal au liquide, car la base de cet organe est séparée de l'extrémité distale des $Mx_2$ par la série des mues larvaires emboîtées; d'autre part, je n'ai pu retrouver, dans les parois du pédoncule, les pores qui, d'après Zandt les cribleraient et par lesquels le liquide sécrété pourrait être évacué et venir enrober d'une masse visqueuse l'axe que formerait ainsi le filament fixateur. A mon sens, le passage de l'appareil larvaire à l'appareil définitif est très difficile à comprendre et si l'explication ingénieuse proposée par Zandt chez l'*A. pseudobasanistes* est exacte, je dois dire que je n'ai pu la vérifier dans tous ses détails chez *S. mattheyi.* J'ai d'ailleurs fait quelques constatations, malheureusement trop peu nombreuses pour me donner une certitude, mais qui suggèrent un tout autre mécanisme: j'ai vu en effet une femelle de dix jours qui avait rompu sa

fixation maxillaire et se déplaçait sur la peau de son hôte. On pourrait supposer qu'il s'agit là d'un processus général et que la fixation définitive interviendrait après relâchement de la fixation larvaire. La femelle, dans cette hypothèse, se cramponnerait à son hôte au moyen de ses Mxp, et, enfonçant alors ses $Mx_2$ sous l'épiderme, construirait le bouton fixateur définitif directement sous la peau du Poisson. Rappelons à ce propos que le bouton est fait d'une substance souple et plastique et qu'il est divisé en deux cavités par une cloison (fig. 1 et 2), chacune d'entre-elles étant en rapport avec un bras maxillaire. Il est intéressant de constater que la longueur du bouton de fixation (1,5 mm.) est double de celle du pédoncule primitif (0,7 mm.). En passant du stade VI au stade VII, l'appareil fixateur n'est pas seul à se modifier, puisque tous les appendices se transforment, à l'exception pourtant des mandibules. Nous ne reviendrons pas sur la description de la femelle au stade VII, ou femelle adulte, car nous lui avons déjà consacré un chapitre spécial.

Quelle est la durée de la vie des *Salmincola* ? A cette question nous avons pu, en ce qui concerne le mâle, répondre d'une manière précise: la mort suit de près l'accouplement, ce qui nous permet d'évaluer à quelques jours l'existence du mâle. Il est beaucoup plus difficile de fixer la longévité des individus femelles. J'ai cependant fait quelques observations qui apportent une solution, partielle tout au moins, au problème posé. Un Omble, infesté au Laboratoire, a survécu un mois; ce Poisson était porteur d'une femelle dont la taille, lorsque mourut son hôte, était encore bien inférieure à celle de l'adulte (pl. 1, fig. 1). Chez un autre Omble, porteur lors de sa capture d'une femelle adulte pourvue de sacs ovigères, la survie au laboratoire fut encore d'un mois; or, la déhiscence des sacs n'avait pas encore eu lieu. D'autre part, l'examen de la cavité abdominale des parasites montre que des femelles, porteuses de sacs ovigères, peuvent renfermer: soit des jeunes cordons ovariens riches en ovogonies, soit de gros ovules en formation, soit enfin de rares ovules disséminés. Ces faits rendent très possible l'hypothèse de deux pontes successives, et ce d'autant plus que le réceptacle séminal, vide dans le troisième cas, renfermait de nombreux spermatozoïdes dans les deux premiers.

Nous pouvons déduire de ces observations que l'incubation des œufs dure plus d'un mois, qu'il y a au moins deux pontes succes-

sives et que les premiers sacs ovigères apparaissent plus d'un mois après la fixation de la larve sur son hôte. La durée de la vie serait donc de trois mois au moins, probablement de quatre à cinq en réalité.

A la suite de cette étude, nous pouvons résumer le cycle évolutif complet de *Salmincola mattheyi* et le comparer avec celui que Zandt a décrit chez *Achtheres pseudobasanistes:*

| *Salmincola mattheyi.* | *Achteres pseudobasanistes.* |
|---|---|
| Stade I libre, puis fixation pédonculaire frontale. | Stade I libre, puis fixation pédonculaire frontale. |
| Stade II, fixation pédonculaire par les $Mx_2$. | Stade II, fixation frontale. |
| Stade III, idem. | Stade III, fixation pédonculaire par les $Mx_2$. |
| Stade IV, idem. | Stade IV, idem. |
| Stade V, idem. | Stade V, idem. |
| Stade VI, différenciation des sexes; les ♀♀ ont conservé la même fixation, les ♂♂ ont retrouvé leur liberté et meurent après l'accouplement. | Stade VI, idem, différenciation des sexes. |
| Stade VII, ♀♀ adultes; fixation par les $Mx_2$, au moyen d'un bouton. | Stade VII, ♂♂ mûrs et libres, fixation des ♀♀ inchangée. |

L'accouplement au stade VI est caractéristique de notre espèce; la petitesse des parasites à ce stade nous explique le fait que dans de nombreuses espèces se rattachant à la famille des *Lernaeopodidae*, les mâles, difficiles à découvrir, sont encore inconnus; nous n'avons pas eu le bonheur d'en trouver un seul, sur quelques 300 Ombles-Chevaliers observés. Si nous songeons à la durée relative de la vie dans les deux sexes, la chance de trouver un mâle est extrêmement faible, et ceci d'autant plus qu'ils ne mesurent qu'un millimètre environ, qu'ils sont translucides et qu'ils peuvent se détacher du Poisson au cours de la manipulation, si méticuleuse que puisse être cette dernière.

### 4. La biologie larvaire.

Les conditions de vie de *S. mattheyi* sont liées à celles de l'Omble chevalier qu'il parasite: dans le Léman, l'Omble est un Poisson des grands fonds qui ne remonte qu'exceptionnellement au-dessus de 30 mètres; d'après les observations des pêcheurs, et comme nous l'avons déjà mentionné, il est toujours localisé entre 30 et 100 mètres. Dans ces zones, la température de l'eau est basse, variant entre

+5° et +11°; l'obscurité est pratiquement totale au-dessous de 40 mètres. Cette localisation de l'Omble dans les eaux profondes doit s'expliquer en partie par l'énergique phototactisme

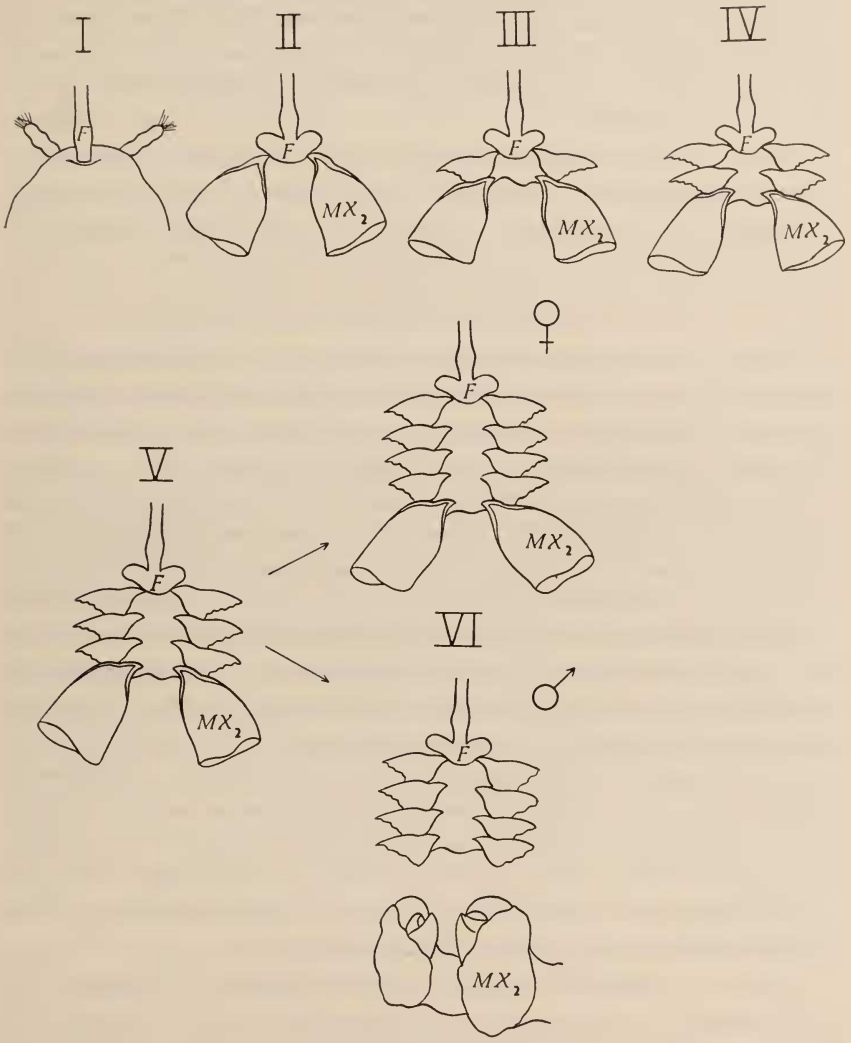


Fig. 53.

Schéma montrant les rapports de l'extrémité distale des $Mx_2$ avec la partie basale du filament fixateur, au cours de l'évolution larvaire et dans les deux sexes. Remarquer l'emboîtement des exuvies.

négatif que ces Poissons présentent et qu'il est facile de mettre en évidence en abandonnant à la surface du lac des alevins jusqu'alors élevés à la lumière. Ces jeunes Ombles descendent directement vers le fond. Or, le parasite, sous sa forme infestante, étant au contraire doué d'un phototactisme positif énergique qui tendrait à l'éloigner de l'hôte, il ne pourrait se reproduire dans les eaux éclairées (cf. p. 45). Il est fort possible que ces considérations donnent une explication tout au moins partielle de la spécificité parasitaire de notre Copépode.

Pour réunir les faits sur lesquels nous avons basé notre étude, la reconstitution du cycle évolutif du parasite au laboratoire a été nécessaire, et nos techniques finirent par tendre à la reproduction plus ou moins fidèle des conditions du milieu naturel.

### Les conditions de l'éclosion.

Nous ne reviendrons pas sur la description de l'éclosion proprement dite que nous avons déjà traitée dans le précédent chapitre, mais nous parlerons seulement des conditions biologiques indispensables à ce phénomène. Pour obtenir l'éclosion d'œufs au laboratoire, on choisit des femelles fraîches de *S. mattheyi* portant des sacs ovigères mûrs, qu'on reconnaît à leur teinte grisâtre. On détache ces sacs en les coupant au ras du corps. Il faut faire attention de ne pas entailler la membrane du sac, car l'eau pénètre alors à l'intérieur; les œufs se gonflent par osmose avant terme et peuvent être considérés comme perdus. La réussite de l'incubation dépend avant tout de la température à laquelle on opère: en hiver, durant les mois de novembre, décembre, janvier, février et mars, la technique est simple: les sacs ovigères sont placés dans des cristallisoirs remplis d'eau qu'on installe en plein air, dans un endroit à l'abri du vent, du soleil et du gel; on obtient ainsi facilement une température de $+4°$ à $+12°$; si des poussières s'accumulent dans les cristallisoirs, il est préférable de changer fréquemment l'eau, afin d'obtenir des larves dans un milieu propre.

Après une durée qui varie suivant le degré de maturité, les œufs éclosent; mais si après une quinzaine de jours l'éclosion ne s'est pas produite, c'est que les sacs étaient trop jeunes; l'incubation de tels sacs aboutit régulièrement à un échec. Il est intéressant de remarquer, que les deux sacs ovigères de la même femelle, placés dans les mêmes conditions de température, éclosent en

même temps. Les larves ainsi obtenues sont très agiles et vivent environ vingt-quatre heures. Dès les beaux jours du mois d'avril, la température s'élève déjà trop, les œufs n'éclosent plus. Nous avons alors essayé de mettre les sacs ovigères à l'eau courante ou dans un cristallisoir refroidi par un courant d'eau; mais la température de l'eau de Lausanne étant à cette saison de 14°, les résultats furent médiocres. Nous avons obtenu de temps à autre une éclosion, mais les larves produites sont peu agiles, vivent quelques heures seulement et sont trop faibles pour se fixer sur un Omble. Nous avons aussi essayé de mettre incuber nos sacs ovigères dans une armoire frigorifique; les résultats ne furent pas brillants. Une basse température (0 à + 3°) augmente par trop la durée d'incubation, les embryons meurent pour la plupart et les larves qui naissent sont faibles. Ne pouvant disposer en été d'une température constante entre + 4° et + 11°, et pour éviter de construire un appareil qui m'aurait donné ces températures mais qui aurait été difficile à mettre au point, je me suis décidé à poursuivre mes essais dans le milieu naturel de *Salmincola mattheyi*, c'est-à-dire au fond du lac. Cette technique me prit beaucoup de temps, mais d'excellents résultats me récompensèrent. Voici comment j'ai procédé: au large de Rolle, près de la station de pêche des Ombles, j'ai ancré une bouée et sur le filin de 40 mètres reliant le corps mort à l'indicateur flottant de la surface, j'attachais par 30 mètres de fond des éprouvettes, fermées avec de l'organdi, et qui contenaient des sacs ovigères à incuber; afin de ne pas laisser échapper une éclosion, chaque matin et chaque soir, alors qu'une fraîcheur relative règne sur le lac, j'allais remonter à la surface ma « station d'élevage »; les larves ainsi obtenues sont robustes; conservées une nuit à la glace dans un thermos elles demeurent très agiles le lendemain et capables de se fixer sur un Omble; nous avons pu déterminer par cette méthode que les larves vivent trois jours au fond du lac, alors qu'au laboratoire, dans les mêmes conditions de température, nous n'avons jamais pu les garder vivantes plus de vingt-quatre heures.

### Le Phototactisme.

Comme nous l'avons déjà mentionné, les larves nageuses sont douées d'un phototropisme si énergique, qu'en milieu éclairé, elles n'obéissent qu'aux excitations lumineuses.

Nous avons procédé sur ce point à des expériences simples, mais qui n'en sont pas moins démonstratives. Voici la technique: nous avons pris un tube de verre de 15 cm. sur 1,5 cm. de diamètre, rendu opaque sur toute sa longueur, les deux extrémités restant seules transparentes; nous avons introduit des larves nageuses très agiles dans l'eau de ce tube; en l'immergeant fréquemment dans de la glace fondante, nous maintenons les larves à leur température optimale. Remarquons qu'à température trop élevée (au-dessus de 11°) les larves deviennent indifférentes aux excitations lumineuses, que leurs mouvements se ralentissent et que la mort s'en suit si cet état se prolonge. Notre méthode empirique consiste à amener, par l'action de la lumière blanche, toutes les larves à une extrémité du tube. Si nous transportons ce dernier à l'obscurité, les larves ainsi groupées mettent trois à quatre minutes pour se répandre uniformément dans toute sa longueur, tandis que si nous éclairons maintenant uniquement son autre extrémité, toutes les larves le traversent et viennent se réunir vers la partie éclairée. Nous avons observé les réactions des larves pour différentes gammes chromatiques: les larves sont premièrement amenées à une extrémité du tube au moyen de lumière blanche, puis nous observons si une autre radiation est capable de les faire traverser le tube. Nous n'avons pu, faute d'un équipement suffisant, donner à ces observations la précision exigée pour des mesures physiques: d'une façon générale, la lumière blanche est plus active que les radiations monochromatiques, parmi lesquelles l'effet le plus intense est obtenu avec les courtes longueurs d'ondes: les larves, excitables, par le bleu, le vert, le jaune et l'orangé, ne réagissent plus au rouge.

Ces indications, d'ordre purement qualitatif, ne tiennent pas compte de l'intensité lumineuse; j'ai dû travailler avec une instrumentation de fortune: lampes et écrans colorés, dont les caractéristiques physiques n'étaient pas définies. Cependant, certaines observations montrent clairement que l'importance de la longueur d'onde est plus grande que celle de l'intensité; celle-ci, singulièrement un verre de cobalt épais, ne laissant filtrer que peu de lumière, agit aussi énergiquement sur les larves que l'illumination, relativement brillante, issue d'une lampe jaune. Je me réserve d'ailleurs de reprendre ces recherches dès que je pourrai disposer de l'outillage nécessaire. Je ferai encore remarquer que dans

les profondeurs du lac où vivent l'Omble et son hôte, l'énergie lumineuse est très faible et limitée précisément à ces radiations de courtes longueurs d'ondes, auxquelles les larves de *Salmincola mattheyi* sont sensibles. Puisque à la profondeur où évoluent nos parasites, la fixation n'est pas influencée par le phototactisme, il faut admettre que l'intensité lumineuse est déjà si affaiblie qu'elle se trouve en dessous du seuil de perception.

Fasten a constaté chez la larve infestante de *Salmincola edwarsii* un phototropisme positif pour une forte intensité lumineuse seulement et a basé sur cette constatation un ingénieux procédé pour lutter contre l'infection des viviers à truites par ce parasite: il éclaire à la lampe à arc et récolte au moyen d'un filet de gaze les larves attirées à la surface. Une autre larve infestante de *Lernaeopodidae* très phototropique est celle de l'*Achtheres pseudobasanistes*, citée par Zandt.

## L'infestation.

En raison de leur phototropisme, les larves, dans les conditions expérimentales, ne vont se fixer sur leur hôte que dans une obscurité pratiquement profonde : la technique d'infestation consiste de nouveau à placer le parasite et son hôte dans un milieu semblable au milieu naturel. Nous avons introduit à l'obscurité des larves nageuses dans un bac de deux à trois litres d'eau contenant un Omble. La température de cette eau est maintenue basse en ajoutant de la glace ou en plaçant le bac dans un frigorifique; après cinq ou six heures, la majorité des larves étant fixées sur leur hôte et ne risquant plus d'être entraînées, nous rendons au Poisson l'eau courante indispensable. Mais, comme j'ai pu le constater, les larves déjà fixées sont encore capables d'obéir à leur phototropisme et de relâcher leur fixation, tant qu'elles ne sont pas métamorphosées en deuxième stade copépodite, ce qui correspond à la perte des appendices natatoires; nous devons alors, par mesure de sécurité, maintenir le Poisson infesté pendant deux jours à l'obscurité.

Il est intéressant de constater la répartition des larves fixées sur tout le corps du Poisson; au début de la fixation, elle est très uniforme, puis de jour en jour disparaissent les larves fixées sur les parties du corps de l'hôte les plus exposées aux frottements avec les parois de l'aquarium, de sorte qu'après cinq ou six jours,

des larves ne s'observent plus que sur les nageoires pectorales, sur la partie des opercules protégée par ces nageoires, sur le dos et sur les nageoires dorsales et ventrales. Lors de la capture des *Salmincola mattheyi* sur les Ombles du Léman, nous avons établi la répartition (fig. 54) moyenne des parasites à la surface du corps; la statistique obtenue se rapproche de la précédente, mais, en aqua-

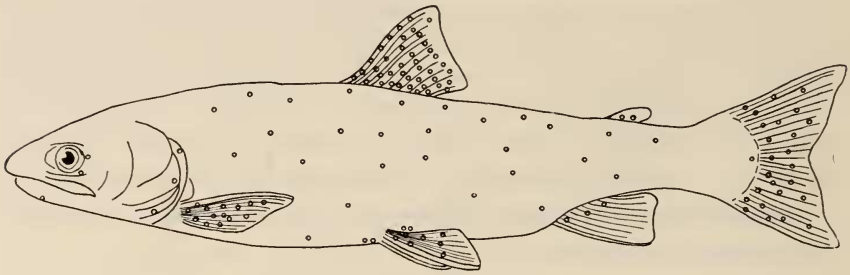

Fig. 54.
Schéma représentant la répartition moyenne des parasites sur l'Omble.

rium, la partie caudale du poisson est en perpétuel frottement avec les parois, ce qui la débarrasse de ses larves; dans la nature, les *Salmincola* abondent autant sur la queue que sur les autres nageoires; les flancs et la tête sont par contre beaucoup moins parasités que les nageoires et la zone périphérique qui les porte.

Nous avons infesté des Ombles de 20 à 25 cm. et des alevins d'Ombles. Pour prélever les parasites à différents âges chez l'hôte adulte, nous devions couper, chez le Poisson vivant, le fragment de nageoire porteur de larves, tandis qu'en opérant avec des séries d'alevins, il nous suffisait de fixer tout entier l'alevin infesté. Grâce à ces diverses techniques, nous avons pu observer le développement dans les 15 premiers jours.

Nous nous sommes ensuite demandé si notre Copépode parasite était spécifique de l'Omble-Chevalier et pour trancher la question, nous avons essayé de le mettre en présence d'autres espèces de Poissons; nous avons réussi à infester très facilement des *Trutta fario* L. de 10 cm., qui ont malheureusement péri quelques jours après l'infestation; cependant nous avons ainsi appris que *Salmincola mattheyi* se développe normalement sur la Truite jusqu'au stade V. Il est curieux de constater l'extrême sensibilité de la Truite

aux petites lésions cutanées provoquées par la fixation du parasite. Nous sommes en effet enclins à croire que ces Poissons sont morts directement des suites de l'infestation, car les témoins vivent très longtemps au laboratoire. Si dans la nature, *S. mattheyi* ne se rencontre pas sur la Truite, c'est que cette dernière habite des eaux éclairées, condition fatale à la transmission de notre parasite.

Nous avons essayé en vain d'infester un représentant des *Percidae*, *Perca fluviatilis*, et un représentant des *Cyprinidae*, *Phoxinus laevis;* nous n'avons pu obtenir la fixation de larves infestantes sur ces espèces.

Nous avons l'intention de revenir prochainement sur cet intéressant problème de la spécificité parasitaire.

## CONCLUSIONS

Je terminerai ce travail par un bref résumé de mes recherches.

Sur les Ombles-chevaliers du Léman, se rencontre très fréquemment un Copépode cuticole, qui, chose étrange, n'avait jamais été décrit jusqu'ici. En dehors de caractères morphologiques bien nets, l'espèce que j'ai nommée *Salmincola mattheyi*, se distingue de tous ses congénères par le fait qu'il ne se fixe jamais sur les branchies, mais uniquement sur la peau et les nageoires de son hôte.

J'ai pu suivre le développement de cet animal et obtenir au laboratoire l'infestation expérimentale de l'Omble. De l'œuf sort la larve copépodite nageuse qui se fixe sur son hôte au moyen d'un appareil lequel est frontal tout d'abord, puis maxillaire. La femelle demeure définitivement reliée à son hôte, alors que le mâle reprend sa liberté. Au bout de dix jours, la détermination sexuelle est accomplie et la copulation a lieu, acte auquel le mâle ne survivra que peu d'heures. La femelle, au contraire, subsiste plusieurs mois.

Le développement est accompagné de mues dont j'ai pu établir le nombre et la durée exacte, pour les deux sexes. J'ai pu enfin décrire avec beaucoup de précision l'organisation du parasite à tous les stades de son existence.

## LISTE DES ABBRÉVIATIONS

| | |
|---|---|
| A | Anus. |
| $A_1$ | Antennule. |
| $A_2$ | Antenne. |
| B | Bulbe buccal. |
| BF | Bouton fixateur. |
| C | Canal efférent de la glande sécrétrice G. |
| CD | Canal déférent. |
| CE | Canal excréteur maxillipédique. |
| CG | Carrefour génital. |
| E | Estomac. |
| EC | Ebauche des canaux génitaux. |
| F | Filament fixateur. |
| FC | Fossette ciliée. |
| G | Glande sécrétrice œsophagienne. |
| GC | Glande cémentaire. |
| GE | Glande maxillipédique excrétrice. |
| GF | Glande frontale. |
| GN | Ganglion nerveux périœsophagien. |
| GO | Gonade. |
| GS | Glande sécrétrice du filament. |
| I | Intestin. |
| LI | Lèvre inférieure. |
| LS | Lèvre supérieure. |
| M | Muscles stomacho-pariétaux. |
| Me | Membrane frontale. |
| Md | Mandibule. |
| MG | Masse glandulaire. |
| $Mx_1$ | Première maxille. |
| $Mx_2$ | Deuxième maxille. |
| Mxp | Maxillipède. |
| NA | Nerfs antérieurs. |
| NO | Nerf optique impair. |
| NP | Nerf postérieur impair. |
| NV | Nerfs ventraux. |
| O | Ovaire. |
| Oc | Œil. |
| Oe | Œsophage. |
| OP | Orifice de ponte. |
| OS | Orifice sexuel. |
| $Pr_1$ | Premier péréopode. |
| $Pr_2$ | Second péréopode. |
| R | Rectum. |
| RS | Réceptacle séminal. |
| S | Sphincter. |
| SO | Sac ovigère. |
| Sp | Spermatophore. |
| T | Testicule. |
| V | Vagin. |

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## EXPLICATION DE LA PLANCHE 1

*Salmincola mattheyi* n. sp.

Fig. 1. — Jeune femelle; début du stade VII.
Fig. 2. — Accouplement; le mâle, à la partie inférieure du cliché, dépose ses spermatophores, au moyen de ses Mxp sur les orifices de ponte de la femelle.
Fig. 3. — Femelle adulte avec ses sacs ovigères.

TABLE I

SUMMARY OF HCN DETERMINATIONS

| Exp. No. | Flax No. | Height (inches) | Age | Per cent HCN in flax strain Number | | | | | | | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 11 | |
| 29 | 1 | | 6½ wks. | .18 | | | | | | | | |
| 31 | 7 | | 7 wks. | | | | | | | .12 | | Shoots |
| | | | | | | | | | | .07 | | Roots |
| 33 | 4 | 5½–6 | 8 wks. | | | | .12 | | | | | Whole plants |
| 34 | 2 | 7½–8½ | 8 wks. | | .11 | | | | | | | Whole plants |
| 37 | 6 | 5½–6 | 8 wks. | | | | | | .097 | | | Whole plants |
| 40 | 1 | 2½ | 5½ wks. | .12 | | | | | | | | High dry weight. Whole |
| 42 | 6 | 4 | 6 wks. | | | | | | .066 | | | Whole plants |
| 42 | 5 | 5½–6 | 6 wks. | | | | | .08 | | | | Whole plants |
| 42 | 3 | 3½–4½ | 6 wks. | | | .075 | | | | | | Whole plants |
| 42 | 2 | 4½–5 | 6 wks. | | .085 | | | | | | | Whole plants |
| 43 | 2 | 3–3½ | 2 wks. | | .083 | | | | | | | Constant-light room |
| 43 | 6 | 3–4 | 2 wks. | | | | | | .11 | | | Constant-light room |
| 57 | 3 | 10–11 | 11 wks. | | | .045 | | | | | | |
| | | 7½–8 | | | | .078 | | | | | | From same flat |
| 60 | 1 | 10–11 | 11 wks. | .20 | | | | | | | | Tops, 2–4 inches |
| 61 | 1 | Seedlings | 5 days | .28 | | | | | | | | Stems. In dark |
| | | | | .15 | | | | | | | | Roots. In dark |
| 62 | 2 | 3–3½ (2–6½) | 15 days | | .054 | | | | | | | Constant light. Poorly watered. Irregular height |
| 62 | 6 | 4(2–7) | 15 days | | | | | | .046 | | | Constant light. Poorly watered. Irregular height |
| 64 | 6 | 10–14 | 8 wks. | | | | | | .086 | | | Low-temp. house, 52° C. night |
| 64 | 6 | 7–11 | 8 wks. | | | | | | .094 | | | High-temp. house, 78° C. |
| 66 | 2 | 10–14 | 8 wks. | | .061 | | | | | | | Low-temp. house |
| 66 | 2 | 7–11 | 8 wks. | | .069 | | | | | | | High-temp. house |
| 68 | 1 | 8½–10 | 6½ wks. | .031 | | | | | | | | Low-temp. house |
| 68 | 2 | 8–9½ | 6½ wks. | | .085 | | | | | | | Low-temp. house |
| 69 | 1 | 9½–12 | 7 wks. | .022 | | | | | | | | |
| | | | | .020 | | | | | | | | Checks |
| 70 | 7 | 4–4½ | 3 wks. | | | | | | | .127 | | |
| | | | | | | | | | | .131 | | Checks |
| 73 | 3 | 4–5½ | 3 wks. | | | .059 | | | | | | |
| 74 | 2 | 6–9 | 16 days | | .087 | | | | | | | Constant light |
| 74 | 6 | 7½–9½ | 16 days | | | | | | .19 | | | Plus 2 days ordinary |
| 75 | 2 | Seedlings | 5 days | | .35 | | | | | | | Stems by aeration |
| | | | | | .062 | | | | | | | Roots by aeration |

TABLE I (*continued*)

| Exp. No. | Flax No. | Height (inches) | Age | Per cent HCN in flax strain Number | | | | | | | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 11 | |
| 76 | 2 | Seedlings | 5 days | | .31 | | | | | | | Stems } Juice filtered into KOH |
| | | | | | .051 | | | | | | | Roots } |
| 77 | 4 | 3½–4½ | 3 wks. | | | | .11 | | | | | 59° C. at night |
| | 6 | 3½–4½ | | | | | | | .089 | | | |
| 79 | 2 | 3–4 | 16 days | | .19 | | | | | | | |
| 82 | 2 | 7–8 | 30 days | | .053 | | | | | | | |
| | 6 | 7½ | | | | | | | .06 | | | |
| 83 | 1 | 1½–2 | 3 wks. | .08 | | | | | | | | Aeration faulty |
| 83 | 4 | 1½ | 3 wks. | | | | .13 | | | | | |
| 85 | 2 | 4–5 | 19 days | | .18 | | | | | | | |
| | 5 | 4½–5½ | | | | | | .061 | | | | |
| 87 | 1 | 3–4 | 30 days | .034 | | | | | | | | Slow-growing in cold-frame |
| | 1 | 20–22 | 7–8 wks. | Trace | | | | | | | | |
| 90 | 4 | 3–5 | 5 wks. | | | | .073 | | | | | |
| 91 | 1 | 2½–3 | 3 wks. | .015 | | | | | | | | |
| | 6 | 2½–3 | | | | | | | .011 | | | In cold-frame |
| 99 | 1 | 4½–5 | 4 wks. | .066 | | | | | | | | |
| | 6 | 3–4 | 4 wks. | | | | | | .056 | | | In cold-frame |
| 100 | 1 | 6–8 | 6 wks. | .019 | | | | | | | | In cold-frame |
| 104 | 4 | 7½–9 | 6 wks. | | | | .029 | | | | | |
| 108 | 2a | 5–6 | 5 wks. | | .063 | | | | | | | Healthy |
| | | 3–4 | | | .0083 | | | | | | | Yellow |
| 109 | 6 | 4½–6 | 5 wks. | | | | | | .078 | | | |
| 112 | 1 | 4½–6½ | 7 wks. | .017 | | | | | | | | |
| | 3 | 5–6 | | | | .009 | | | | | | |
| 113 | 1 | 5–7 | 7 wks. | .006 | | | | | | | | Duplication of No. 112 but 2 days later |
| | 3 | 6–7 | & 2 days | | | .01 | | | | | | |
| 114 | 2a | 6½–11 | 7 wks. | | .01 | | | | | | | |
| | 6 | 6½–8 | | | | | | | .012 | | | |
| 125 | 2a | Seedlings | | | .094 | | | | | | | Possible loss during storage |
| | 7a | Seedlings | | | | | | | | .027 | | |
| 128 | 11 | 2–4 | 2 wks. | | | | | | | | .243 | |
| 136 | 1a | 3–3½ | 12 days | .11 | | | | | | | | |
| 145 | 11 | 5–10 | 5–6 wks. | | | | | | | | .20 | |

Note:—Nos. 1a and 2a a different year's production of seed; No. 11 a highly resistant selection from No. 1.

TABLE II

THE GENERAL COURSE OF HCN CONTENT OF FLAX THROUGHOUT THE LIFE OF THE PLANT

| Height of flax plant in inches | Per cent HCN found | Per cent glucoside calculated |
|---|---|---|
| Seed | 0.008 | 0.07 |
| 1–1.5 | .15 | 1.4 |
| 2–3 | .17 | 1.5 |
| 3–4 | .15 | 1.4 |
| 4–5 | .13 | 1.2 |
| 5–6 | .10 | 0.9 |
| 6–7 | .10 | 0.9 |
| 8–9 | .08 | 0.7 |
| 12–15 | .07 | 0.6 |
| 15–18 | .03 | 0.3 |
| 18 | 0.009 | 0.08 |
| 18 | None | None |

for even a day or so may influence this quantitative characteristic immediately (Exps. 112 and 113). Willaman and West ('16) noted some climatic effect on the production of HCN by sorghum, although they were inclined to believe that varietal constitution caused greater modifications than did climatic differences. Pinckney ('24), however, was convinced that HCN was increased in direct proportion to the increase of nitrate fertilizer on a low nitrate field, and that yellowish sorghum produced little if any HCN when the green plants contained a good supply.

While in general young flax runs high in hydrocyanic glucoside and old flax low, yet it appears that neither age nor height as such are proper indicators of the probable HCN production. The condition which seems to influence this quantitative factor is the number of actively functioning cells. Thus plants of the same age, but of different physiologic vigor, may develop nearly eight times as much HCN in the normal healthy specimens as in yellow retarded ones (Exp. 108). Experiments 43 and 62, although originally intended to be duplicates, carried on in the constant-light room, do not show the same quantities of HCN. In the latter experiment, due to the crowded condition of the room, the flax flats were irregularly and inadequately watered. The resulting plants, although of equal age, were very unequal in size and vigor of growth. The amounts of HCN in both strains were noticeably less than in Experiment 43 in which the growth was

uniform and normal. In the examples cited the irregular watering of the flats seemed to be the factor which largely determined the relative vigor of the plants. The upper two to four inches of flax eleven weeks old, which was in vigorous physiologic condition, gave a high quantity of HCN hardly equalled even by very young flax (Exp. 60). Thus it would appear that the reason the percentage of HCN in flax shows a steady decrease from a maximum at 2–4 inches high is because a continually decreasing proportion of the plant is composed of actively functioning cells.

Temperature variations of medium range did not seem to modify the glucoside content (Exp. 64 and 66). The use of the constant-light room did not seem to cause any special change in the quantity either, although the life cycle was greatly shortened so that seed was matured in about half the usual time. The shoots of flax seedlings may contain five times as much glucoside as the roots (Exps. 75 and 76), while in older plants there is a smaller difference (Exp. 31), due mainly perhaps to the drop in the percentage in the shoot. In the older plants it is very difficult to get the smaller and hence physiologically more active roots for a quantitative determination, so that it is not known at present how much glucoside is in the whole root system. It is interesting to note that while fully mature flax seed contains very little, if any, glucoside which may be converted into HCN, five-day-old seedlings, which had developed in the dark, had the highest percentage of the acid which was found in this series of experiments (Exps. 61, 75, and 76).

The flax strains numbered 1, 2, 3 and 4 are, in the main, strongly resistant to the wilt disease, while No. 5 is intermediate and Nos. 6 and 7 are only slightly, if at all, resistant, as determined through field tests by Professor Bolley. Although in many of the analyses the more resistant varieties ran high in glucoside content and the less resistant ones low (table III) not enough data were gathered to prove definitely a correlation (Exps. 42, 77, 85, etc.). It may be noted from table III that the 2–6 pair, which in four out of seven tests showed a greater amount of HCN in the susceptible flax than in the resistant, accounts for four out of the nine cases in which this same relationship is seen. Under most conditions the No. 6 flax seems to produce more HCN than No. 2.

It may be that it has more glucoside or that under certain circumstances it produces a larger amount of linase, or a more active enzyme. Strain 4 consistently developed a high quantity of HCN for the conditions under which it was growing and constitutes the other apparent exception to a correlation between strong resistance and high glucoside content. However, Professor Bolley's characterization of its degree of resistance indicates that it is rather likely to be exceptional.

TABLE III

PAIRS OF FLAX VARIETIES COMPARED AS TO RELATIVE QUANTITIES OF HCN. R = MORE RESISTANT MEMBER OF PAIR; S = MORE SUSCEPTIBLE

| Exp. No. | Pairs of varieties | R more HCN than S | S more HCN than R |
|---|---|---|---|
| 42 | 5–6 | * | |
| | 2–3 | * | |
| | 2–6 | * | |
| | 3–6 | * | |
| | 3–5 | | * |
| | 2–5 | * | |
| 43 | 2–6 | | * |
| 62 | 2–6 | * | |
| 68 | 1–2 | | * |
| 74 | 2–6 | | * |
| 77 | 4–6 | * | |
| 82 | 2–6 | | * |
| 83 | 1–4 | | * |
| 85 | 2–5 | * | |
| 91 | 1–6 | * | |
| 99 | 1–6 | * | |
| 100, 104 | 1–4 | | * |
| 112 | 1–3 | * | |
| 113 | 1–3 | | * |
| 114 | 2–6 | | * |
| 125 | 2–6 | * | |

It is evident from Broadfoot's work ('26) that each of the different strains of flax is resistant to the different strains of *Fusarium lini* in different degrees. Because of these considerations and of the variety of conditions surrounding the plants and the ease with which the glucoside content may change we must conclude that the lack of an evident, exact correlation does not disprove a possible causal relationship between resistance and glucoside production. Many more determinations of the glucoside and of the changes in amounts of linase in flax must be made before a clear

picture of the distribution and significance of HCN can be drawn. Such a study is planned for the near future.

While it had been determined formerly (Reynolds, '24) that potassium cyanide inhibits the growth of *Fusarium lini* on agar plates at a concentration of about .03M it was thought best to test the effect of cyanide in liquid cultures. The following experiment (No. 15) was made: A solution of hydrocyanic acid was prepared so that 5.2 cc. in a 50-cc. culture would give a .03M concentration of HCN. The standard culture medium described in the third section of the paper was used and a series of cultures made as indicated in table IV. Some of these were inoculated with No. 2 *Fusarium lini* and some with No. 7 *Fusarium lini*. The former was one of Broadfoot's ('26) most virulent flax parasites and the latter one of the least parasitic of his group. The cultures in the Florence flasks (F) were sealed with paraffin to

TABLE IV

EFFECT OF CYANIDE IN LIQUID CULTURES

| *F. lini* culture | Amount of HCN sol. (cc.) | Style of flask | Average growth in milligrams of dry weight at | | |
|---|---|---|---|---|---|
| | | | 15° C. | 20° C. | 27° C. |
| # 2 | 0.2 | F | *3—(4) | 3—188.8 | 3—122.0 |
| # 7 | 0.2 | F | 3—229.7 | 3—347.6 | 3—233.9 |
| # 2 | 1.0 | F | 3—(6) | 3—143.3 | 3— 90.0 |
| # 7 | 1.0 | F | 3—(1) | 3—188.7 | 3—(2) |
| # 2 | 5.2 | F | 2—none | 2—none | 3—none |
| # 7 | 5.2 | F | 1—none | 3—none | 3—none |
| # 2 | 1.0 | E | 2—(5) | 2— 48.0 | 2— 75.1 |
| # 7 | 1.0 | E | 1—Trace | 2—222.3 | 2—300.7 |
| # 2 | 5.20 | E | 2—none | 2—(7) | 2— 36.6 |
| # 7 | 5.20 | E | 2—none | 2—(7) | 2—(3) |
| # 2 | None | E | | 3—222.9 | |
| # 2 | None | F | | 3—266.1 | 2— 89.9 |
| # 7 | None | F | 2—343.4 | 2—260.2 | |
| # 7 | None | E | 3—220.7 | | |

* The number preceding the dash indicates the number of cultures in the set. A number in parentheses refers to the following notes.

Notes:
(1) Two with possibly a trace of growth, and third no growth.
(2) One with undetermined amount of growth; one, trace; one, none.
(3) One culture, 31.4 mg.; one, trace.
(4) One culture, 33.9 mg.; one, 7 mg.; one with undetermined amount.
(5) One culture, 30.3 mg.; one, trace around inoculum.
(6) One culture, 21.4 mg.; two, undetermined amounts.
(7) One culture, trace; one, none.

prevent, as far as possible, the escape of HCN. The Erlenmeyer flasks (E) were closed only with cotton plugs.

It will be seen from the results tabulated that the greatest concentration of HCN is practically always completely inhibitive except at the higher temperatures in the non-sealed flasks. The irregular results here indicate that enough of the HCN was lost by diffusion into the air to reduce slightly the toxic quality. This is indicated somewhat also by the observation that nearly all of the HCN flasks in the 27° C. incubator which showed growth were on the lower shelf nearest the heating unit. It appears that the fungus was less resistant to the toxic effect of the HCN at 15° C. than at 20° C., as one would expect from the fact that the former temperature is not as favorable for this species as the latter. The irregular numerical results, especially of the higher concentrations, are characteristic of cultures which are near the inhibitive, toxic concentration of poisons. The No. 2 strain of *F. lini* is a less vigorous grower in this standard medium than the No. 7 strain, but it would seem that there is little difference between the two under the action of the HCN in culture.

The results of the analyses given in table 1 show that in the older flax roots the proportion of HCN to dry weight is much lower than in the shoot. However, they also indicate that in young roots, as found in seedlings, the percentage of HCN may rise to a considerable amount. It is stated by Armstrong and Armstrong ('10) that the entrance of certain substances into a plant which has a cyanogenetic glucoside causes a "cumulative" change, so that a very small portion of the substance produces a large relative production of HCN. If this is true it is entirely conceivable that the entrance of a fungus might likewise stimulate a concentration of the glucoside at the point of attack. Hence the normal concentration of HCN, as determined by analyses such as reported here, does not necessarily have to indicate a toxic concentration in order to account for resistance.

While the various experimental evidences and the considerations just discussed do not give positive proof either for or against the causal relationship between the presence of the cyanophoric glucoside in flax and a certain varietal resistance to the wilt disease, yet it would seem that at least a part of the resistant

quality may be attributed to this chemical condition. In view, however, of the experiments reported in the third section of this paper it is probable that other toxic conditions exist in flax which may also be related to resistance.

## III. Flax Extracts Toxic to Fungi

Although it had been demonstrated that under certain conditions flax extracts retarded the growth of *Fusarium lini* in culture (Reynolds, '24), it was desired to determine what concentration, if any, might prevent the growth of the fungus. Furthermore, as stated in the foregoing section, it was desired to study the effect of the cyanide content of flax upon the fungous growth. Hence water extracts of both fresh and dried flax were tested as regards toxicity to *Fusarium lini*. These extracts were made by grinding the flax and steeping it in water, usually over night. Various methods of filtering and the effects of these upon the toxic quality of the extracts were tested. None of these methods used prevented the characteristic toxic effects. To the filtered liquid were added salts and glucose in the same proportions as used in the standard check medium (A) throughout this study. The formula for this was water 1000 cc., magnesium sulphate 2 grams, later reduced to 1 gram, calcium acid phosphate 1 gram, potassium nitrate 10 grams, and dextrose 20 grams. A portion of each flax extract thus provided with standard quantities of nutrients was autoclaved at 15–20 pounds pressure for twenty minutes; and a corresponding portion filtered through bacteriological filters. At first the Berkefeld and Mandler filter cylinders were used, but the Seitz filter was later adopted for speed and convenience. Sterilized pipettes and culture chambers provided means of transferring the sterilized medium when necessary.

Through the kindness of Dr. E. C. Stakman seven of the strains of *Fusarium lini* with which Broadfoot ('26) carried on his experiments were made available. These were kept in culture and used during the course of the work. They were numbered from 2 to 8 approximately in their general decreasing order of pathogenicity, although it is clear that when several strains of flax are tested the flax strains and those of the fungus can not be arranged in a simple series in relation to one another. Those having the

higher numbers, especially 7 and 8, grew more abundantly in culture media than those having the lower numbers. Many hundreds of cultures were made and the dry weight of growth produced in fourteen days was determined. In nearly all cases identical triplicate cultures were run and averaged for the dry weights. Checks on the standard medium were carried with each set of new inoculations. Flax extracts were made from nearly all of the flax varieties and conditions of growth which were tested for hydrocyanic acid as reported in the preceding section. At the same time that each strain of flax was being tested for HCN content, culture series were run, using both fresh extracts and the extract which had been aerated and hence deprived of most of the HCN. The percentage dry weight of each sample of flax was determined before it was used in the HCN and culture studies. This was necessary since different ages and conditions of growth were being tested. In the course of the aeration process foaming sometimes took place, and at different times diphenyl ether, amyl alcohol, and caprylic alcohol were used to break the foam. Each of these was tested a number of times in different ways as to its effect on the quantity of growth of the fungus. Neither amyl alcohol nor diphenyl ether showed any repressive effect on the fungus and the very dilute quantities used did not stimulate growth. Caprylic alcohol proved to be extremely toxic so that one or two drops in a 50-cc. culture prevented growth completely.

In table v a summary of a considerable number of representative experiments is given. The standard concentration of flax extract was 9 parts water to 1 part dry weight of flax. From .2 to .3 of a gram was the usual dry weight of mycelium produced in the standard check medium A. It was soon evident that dilute flax extracts, that is, below one-half standard strength prepared as stated above, usually stimulated the growth of this *Fusarium*. From .3 to .5 of a gram of growth was usual and the higher the concentration of flax extract, up to certain limits, the greater was the mycelial growth. This favorable effect of the flax medium can probably be ascribed to the added nutritive materials from the flax. Fresh, green flax and dry flax powder

## TABLE V

### RESULTS OF CULTIVATION OF F. LINI IN FLAX EXTRACTS

| Exp. No. | Flax No. and height in inches | Concentration | Filtered (F) Autoclaved (A) | Previous treatment | Result | Remarks |
|---|---|---|---|---|---|---|
| B # 1 | # 6—3½ | 1 | A | Powdered | O | Shoot* |
| B # 1 | # 6—3½ | 1/4–1/2 | A | Powdered | < | Shoot |
| B # 1 | # 6—3½ | 1/2–1 | F | Powdered | O | Shoot |
| B # 1 | # 6—3½ | 1/4–1/2 | F | Powdered | < S | Shoot |
| B # 2 | # 2a—2½ | 1/4–1 | A | Powdered | < | Entire |
| B # 2 | # 2a—2½ | 1/4–1 | F | Powdered | < | Entire |
| B # 3 | # 7—7 | 1/4–1 | A | Powdered | < | Entire |
| B # 3 | # 7—7 | 5/12–1 | F | Powdered | O | Entire |
| B # 3 | # 7—7 | 1/4–1/3 | F | Powdered | G | Entire |
| B # 4 | # 5—12 | 1/4–1 | A | Powdered | G | Leaves |
| B # 5 | # 5—12 | 1/4–1 | A | Powdered | G | Stems |
| B # 6 | # 11—6 | 1 | A | Powdered | O | Leaves |
| B # 6 | # 11—6 | 1/4–1/2 | A | Powdered | G | Leaves |
| B # 6 | # 11—6 | 1/4–1 | F. A. | Powdered | G | Leaves |
| B # 7 | # 11—6 | 1/4–1 | F. A. | Powdered | G | Stems |
| 17 | # 2—2 | 00 | F | Powdered | G> | Roots |
| 18 | # 3—6 | 1/3 | F | Powdered | G | Roots |
| 22 | # 5—8 | 1/4 | F. A. | Aerated | G | Shoot |
| 22 | # 5—8 | 1/20 | F. A. | Aerated | G> | Shoot |
| 22 | # 6—7 | 1/3 | F | | O | Shoot |
| 22 | # 6—7 | 1/3 | A | | G | Shoot |
| 22 | # 6—7 | 1/12 | F | | G | Shoot |
| 22 | # 6—7 | 1/12 | A | | G> | Shoot |
| 30 | # 1 | 1/8 | F | Aerated | G | Shoot |
| 35 | # 7 | 1/10 | F | Aerated | G | Shoot |
| 36 | # 4—6 | 1/8 | F. A. | Aerated | G | Whole |
| 38 | # 6—6 | 1/20 | F | Aerated | G | Less than in Exp. 36 |
| 39 | # 2—8 | 1/16 | F. A. | Aerated | G | |
| 41 | # 1—2½ | 1/14 | F. A. | Aerated | G | |
| 53 | # 6 | 1/7 & 1/28 | F | | G | Fresh, green |
| 54 | # 2 | 1/7 & 1/28 | F. A. | Aerated | G | Fresh, green |
| 54 | # 6 | 1/6 & 1/24 | F. A. | Aerated | G | Fresh, green |
| 58 | # 3—7½ | 1/10–1/40 | F | | G-M | Fresh, green |
| 58 | # 3—10 | 1/15–1/60 | F | | G-M | Fresh, green |
| 63 | # 6—10 | 1/10–1/20 | F. A. | | G > | Fresh, green |
| 65 | # 2—10 | 1/8–1/16 | F. A. | | G > | Fresh, green |
| 67 | # 1 & 2—8 | 1/17 | F. A. | | G | Fresh, green |
| 71 | # 7—4 | 1/14 | F. A. | | G-M-S | Fresh, green |
| 72 | # 3—4 | 1/10–1/20 | F. A. | | G | Fresh, green |
| 78 | # 4 & 6—4 | 1/16–1/32 | F. A. | | G | Fresh, green |
| 80 | # 2—4 | 1/10–1/20 | F. A. | | G | Fresh, green |
| 81 | # 2 & 7 | 2, 1, 1/2 | F. A. | | O-G | Fresh, green |
| 84 | # 1 & 4–1½ | 1/5 | F. A. | | G | Fresh, green |
| 86 | # 2 & 5—5 | 1/4 | F. A. | | G | Fresh, green |
| 88 | # 1—3 & 20 | | F. A. | | G | Fresh, green |
| 92 | # 1 & 6—3 | 1/4 & 1/7 | F | | G | Fresh, green |
| 95 | # 2 & 7 | 2, 1, 1/2 | F. A. | | O | |
| 98 | # 1 & 6—4 | 1/3–1/6 | F. A. | | G | Fresh, green |
| 101 | # 7—10 | 2/3–1/3 | F. A. | | G > | Roots & shoots separate |

TABLE V (*continued*)

| Exp. No. | Flax No. and height in inches | Concentration | Filtered (F) Autoclaved (A) | Previous treatment | Result | Remarks |
|---|---|---|---|---|---|---|
| 117 | # 11—10 | 1 | F | | G | Dry roots only |
| 121 | # 4— | 1 | F | | O | Dried flax powder |
| 123 | # 1a—3 | 1 | F | | O | Fresh, green flax |
| 127 | # 4— | 1 | F | | O | Dry, powdered |

*—Unless definitely stated the extract was made from powdered flax.
M—About equal to growth on Med. A.
O—No growth of fungus developed.
S—Small growth, less than on checks in Med. A.
G—Good growth, distinctly better than checks on Med. A.
>—Weight of fungus decreasing with dilution of extract.
OO—Diluted 50–100 times.
<—Weight of fungus increasing with dilution of extract.

have both been used in preparing these extracts, as illustrated in the following tabulations of results from a few experiments.

Experiment 71—73.2 gms. # 7 fresh green flax to 1000 cc. water

| | Filtered | | Autoclaved | |
|---|---|---|---|---|
| | # 2 *F. lini* | # 7 *F. lini* | # 2 *F. lini* | # 7 *F. lini* |
| Full str. extr. | .2975 | .3707 | .2160 | .3619 |
| Half str. extr. | .2286 | .2354 | .2716 | .3482 |
| Checks # 71 & # 72 | .2442 | .2885 | | |

Experiment 72—106.5 gms. # 3 fresh green flax to 1000 cc. water

| | | | | |
|---|---|---|---|---|
| Full str. extr. | .3147 (.0705)* | .3931 (.1046) | .2977 (.0535) | .3697 (.0812) |
| Half str. extr. | .2782 (.0340) | .3323 (.0438) | .2814 (.0372) | .3302 (.0417) |

*The numbers in parentheses show increase in growth in flax extract over the growth in the check medium.

Experiment 78—61.45 gms. # 4 fresh green flax to 1000 cc. water

| | | | | |
|---|---|---|---|---|
| Full str. extr. | .2832 | .3160 | .2569 | .3079 |
| Half str. extr. | .2687 | .3043 | .2579 | .2905 |

77.14 gms. # 6 fresh green flax to 1000 cc. water

| | | | | |
|---|---|---|---|---|
| Full str. extr. | .3045 | .3323 | .2973 | .3318 |
| Half str. extr. | .2704 | .2871* | .2665 | .3017 |
| Checks | .2179 | .2218 | | |

* Had been contaminated and refiltered before inoculation.

Experiment 80—94 gms. # 2 fresh green flax to 1000 cc. water

| | | | | |
|---|---|---|---|---|
| Full str. extr. | .2771 | .3033 | .2850 | .3048 |
| Half str. extr. | .2973 | .2623 | .2799 | .2817 |
| Checks | .2179 | .2218 | | |

Experiment 81—102.0479 gms. air-dry # 2 flax powder to 500 cc. water. 102.2809 gms. air-dry # 7 flax powder to 500 cc. water. # 7 *F. lini* used

| | # 2 Flax | # 7 Flax | # 2 Flax | # 7 Flax |
|---|---|---|---|---|
| Full str. | .0000 | .0000 | .0000 | .0000 |
| Half str. | .0000 | .0000 | .0000 | .0000 |
| Quarter str. | .5013 | .5149 | .5041 | .5156 |
| Checks | .2409 | | | |

The first four experiments tabulated are typical of many which were carried concurrently with those in which the determinations of cyanide content were made. It will be noted that # 2 *F. lini* consistently produced less growth than # 7 on all flax extracts and the check medium. In Experiment 72 a difference in growth between the check cultures and the flax extract cultures shows that the # 2 *F. lini* made less increase of growth than did # 7 *F. lini*. This would indicate that the former strain can make less use of the added nutritives from the flax or else is retarded more by the flax extract than the latter. It seems that the first alternative is more probable since the # 2 *F. lini* does not make as good use of the nutritives in the check medium as does # 7, and at these concentrations of flax extract a retarding action is not evident. Autoclaving seems to have little effect in changing the nutritive qualities of the flax media at these concentrations, for the differences between the growth in the autoclaved and the filtered media are neither great nor regular. In Experiment 80, since there is more flax per liter than in Experiments 71 and 78,

we should expect a larger fungous growth. However, in the main this is not true. It is possible that a slight toxic effect is exhibited here, but certainly the figures are not clearly significant. Experiment 81, however, exhibits a clear case of toxicity for the half- and full-strength flax cultures and as clearly indicates that the quarter strength is more than twice as effective as the check medium in producing growth. This latter concentration corresponds with the half-standard strength. The juice expressed from fresh flax, when used as above without dilution, also prevented growth of the fungus. In different varieties of flax it was found that various concentrations prevented the growth of *F. lini*. It was necessary therefore to attempt to determine the minimum concentration of flax extracts of different varieties which would just prevent growth of the fungus.

Experiment 147 will illustrate the procedure. Dry flax powder (※ 3 flax), weighing 25.34 gms., was steeped with 100 cc. of distilled water for two days. The liquid was pressed out and more water added with successive pressings until 250 cc. of flax extract had been obtained. Since a small meat press was used some water was left in the flax material and the total water added was somewhat more than the 90 per cent, which has been used as the arbitrary standard. The salts and glucose were dissolved in the standard proportions and the medium was then filtered through the Seitz bacteriological filter. With sterile, graduated pipettes a series of cultures was made as follows, and designated "Series A":—Three tubes of Check Medium A, marked O; three tubes of full-strength extract, marked 1; three tubes with 9 cc. of extract, and 3 cc. of Medium A, marked ¾; three tubes with 6 cc. of extract and 6 cc. of Medium A, marked ½; and three tubes with 3 cc. of extract and 9 cc. of Medium A, marked ¼. These were left several days in the incubator to test for freedom from contamination and then inoculated with ※ 7 *Fusarium lini*. Six days after inoculation there was no growth except in the checks (0). At this time one set, from 0–¼, was reinoculated and designated as X. A second set was filtered into fresh tubes, autoclaved, inoculated, and designated Y. A set Z, made up as follows from the third tube of full-strength flax extract of the original set A, was autoclaved and inoculated:—

(1) 2.0 cc. of full-str. extract and 8.0 cc. of Medium A
(2) 1.5 cc. of full-str. extract and 8.5 cc. of Medium A
(3) 1.0 cc. of full-str. extract and 9.0 cc. of Medium A
(4) 0.8 cc. of full-str. extract and 9.2 cc. of Medium A
(5) 0.6 cc. of full-str. extract and 9.4 cc. of Medium A
(6) 0.4 cc. of full-str. extract and 9.6 cc. of Medium A
(7) 0.3 cc. of full-str. extract and 9.7 cc. of Medium A
(8) 0.2 cc. of full-str. extract and 9.8 cc. of Medium A
(9) 0.1 cc. of full-str. extract and 9.9 cc. of Medium A

Nine days after inoculation no fungous growth was present in sets X and Y except in the ¼-strength culture of Y, where the inoculum had become lodged at the surface of the liquid and a slight growth had developed. In set Z some growth had taken place in all the tubes with evidently much less in (1) and (2). Thus the limiting toxicity for complete inhibition was at ¼ strength for the autoclaved material, although a retarding action was manifested in the second tube of set Z. In the filtered extract the toxic limit for complete inhibition was below the ¼ strength as seen in set X.

In Experiment 102 ✻ 3 flax powder made up one-third standard strength prevented the growth of ✻ 7 *F. lini*. In Experiment 121 one-half strength flax extract from ✻ 4 flax also prevented the growth of the same strain of fungus. Full-strength and one flask of half-strength extract from ✻ 1 flax prohibited growth of ✻ 7 *F. lini*. In this experiment ( ✻ 123) fresh, green flax, 3–3½ inches tall, from the outdoor garden was used. Both the inhibiting strengths had a pH of 3.53. A second flask of the half-strength medium produced a growth of .5087 gms., and an average of three cultures in the quarter strength was .3816 gms. The extra-large growth in the one tube of half-strength medium suggests a toxic stimulatory action. Other occasional results of this irregular nature have been noted, especially at or near the point of complete inhibition. Thirty grams of ✻ 4 flax powder in 300 cc. of water were used in Experiment 138, from which a series of dilutions was made as follows: 1/5, 2/5, 3/5, 4/5, 9/10, and 5/5 strength. A regular increasing gradation of growth from the 3/5 concentration downwards, a slight growth in the 4/5, and none above indicates the approximate inhibiting concentration of this material. A considerable number of the early experiments performed in eastern New York indicated a completely inhibiting toxicity of the flax extracts at or below the standard strength, as

illustrated in the summary given above. These included both extracts from dry flax powder and extracts from fresh, green flax plants. When this work was continued at the Missouri Botanical Garden it was found that some flax powders gave this same inhibiting result, while others allowed abundant growth of *Fusarium lini* at this concentration. This was true even when inoculations were made from the same fungous culture at the same time for different flax extracts. After several series of cultures had given these conflicting results a careful check was made of the sources of the various flax powders used. It was found that uniformly those powders derived from plants grown in New York gave the inhibiting action, while those from plants grown at St. Louis failed to show this degree of toxicity. This interesting phase of the problem will be studied further, when a new supply of flax powders from several climatically different regions is available.

It is evident from these experiments that different strains of flax possess the toxic quality in different proportions and that different environmental conditions surrounding the flax determine the concentration in the same strain at different times.

The effect of heat on the toxic material of flax was tested in two ways. Flax powder was autoclaved for one hour at 18 pounds pressure and used for extracts. No growth of *F. lini* occurred in this extract at standard concentration. Inhibiting flax extracts of standard concentration have been autoclaved and tested for growth of the fungus. In most experiments the fungus was still prevented from growing, but occasionally growth developed. The latter cases have been interpreted as indicating a degree of toxicity close to the margin of inhibition and a partial injury of the toxic material by autoclaving in liquid medium.

Efforts were made to eliminate the toxicity by aeration and to transfer such toxicity to Medium A by aerating flax extracts into this medium. The latter experiment was entirely negative in results and the former essentially so. Fresh, green flax of the ✻ 11 strain was ground and aerated twice for a total of about thirty-six hours. The HCN content was .243 per cent. The residual liquid, 240 cc., was provided with the usual salts and glucose in proper proportion and used as a culture medium for ✻ 7 *F. lini*. A retarded development took place in two filtered

cultures and no growth in the third, while the three autoclaved flasks developed a good growth. Since this medium was somewhat less than one-half standard strength it seems improbable that the aeration had any appreciable effect on its toxicity. In Experiment 102, 39 gms. of ※ 3 flax powder, after a preliminary steeping in water, was aerated into 160 cc. of water, to which the salts and glucose were added to convert it into a standard Medium A plus any volatile products from the flax. This was made into three 50-cc. cultures and called A. The flax filtrate of 1000 cc., obtained after the aeration, was divided into several portions. From 500 cc. of it 280 cc. were distilled into a 5 per cent KOH solution for a cyanide determination. This portion (B) was then made up to its original volume; a portion (C) was retained unchanged; and a portion (D) was diluted to half strength. All these were prepared as usual as cultures and one half of the B, C, and D flasks were autoclaved, while the other half were used as filtered material. The following were the growth results in grams from inoculation with ※ 7 *F. lini*:—A, .3066; B-filtered, .5220; B-autoclaved, .4339; C-filtered, no growth; C-autoclaved, .5681; D-filtered, .4515; D-autoclaved, .4588. Autoclaving again seemed to remove the toxic effect. No toxic effect was noted in the Medium A, thus indicating that no volatile toxin was present in appreciable quantity. The process of distillation, however, destroyed the toxic condition as seen by comparing B and C. The original concentration of flax extract was about one-third standard and when diluted to half strength fell well below the inhibiting concentration as seen in the D cultures. Autoclaving makes little difference in this case.

It was thought that perhaps the toxic material was a dialyzable compound. The following experiments were therefore made to test this hypothesis. For the first experiment (※ 121) 47 gms. of ※ 4 flax powder were left in 450 cc. of water for two days and then, after filtering, washed with several portions of water and pressings in a Buechner funnel with suction until a total of 1000 cc. of filtrate were obtained. Four hundred cc. were then dialyzed through a collodion bag, made as usual in a Kjeldahl flask. An electric stirrer was fitted into the neck of the bag, by a rubber stopper, and dialysis for two days in running tap-water was

followed by one more day in running distilled water. There were 500 cc. of liquid present at the end, to which salts and glucose were added. Fifty-cc. flask cultures were made from this and also from the remainder of the flax filtrate which had not been dialyzed, using as always salts and glucose to equal their concentration in Medium A. Half of the flask cultures were autoclaved and half filtered. None of the cultures from the filtered, original filtrate produced growth; the autoclaved, filtrate cultures produced an average growth of .5923 gms.; the dialyzed, autoclaved cultures produced .2177 gms. of fungous growth; and the filtered, dialyzed cultures had an average of .2634 gms. of fungus. As this flax extract was less than half-standard strength and during the process of dialysis considerable precipitation had taken place in the bag it was thought best to check the results carefully. In Experiment 132, 26 gms. of ※ 6 flax powder were used, since the ※ 4 flax was exhausted. The dialyzing was continued with stirring for three days with a total of 260 cc. liquid left at the end. This was filtered with suction and used without dilution as a culture medium. No growth took place in the filtered series of flasks, but a belated growth of fungus did develop in two out of the three of the autoclaved series in a twenty-day period. No numerical results were taken. If dialysis removes any of the toxicity, as seemed to be the case in the first experiment, it is not rapid nor very effective as seen from the results of the second experiment. In the latter test the filtrate was of standard strength and even in the autoclaved series still showed some toxicity both by the belated development and by the development of growth in only two out of the three flasks. Further dialysis experiments are planned covering several strains and ages of flax.

The relative effects of root and shoot extracts were tested out by an experiment ( ※ 101) as indicated in table VI.

No toxic effects are evident at the concentration used and the root extracts provided less nutriment than those from the shoot. The greater growth of ※ 2 *F. lini*, as compared with ※ 7 *F. lini*, in the shoot cultures may indicate an ability to use flax extractives to better advantage, or possibly an ability to withstand a certain toxic action which, although not sufficient to reduce the growth of ※ 7 *F. lini* below the check culture, nevertheless holds back this

TABLE VI

| | # 7 *F. lini* | | # 2 *F. lini* | |
|---|---|---|---|---|
| | Filtered | Autoclaved | Filtered | Autoclaved |
| Root | | | | |
| Full str. | .5611 | | .5472 | .6714† |
| Half str. | .4057 | .4580* | .3957 | .4773* |
| Shoot | | | | |
| Full str. | .7793 | | .8591 | .8675* |
| Half str. | .4536 | .4887* | | .5775* |
| Checks | .2427 | | .2357 | |

* Average of 2 cultures. † One culture only.

latter fungus from making as full a growth as it should. The latter conclusion would be in accordance with the conclusion reached in Experiment 81. The greater growth of # 7 *F. lini* in the check medium, although numerically not clearly significant, is in line with all of the other cultural work with this strain as compared with # 2 *F. lini*.

It is interesting to note from these various experiments that dilutions of flax extract, which are only half as concentrated as the critical concentration for complete inhibition, often not only allow development of the fungus, but actually produce a much more abundant growth than the standard, check medium. Hence it appears that it is necessary for this toxic material to be in a rather concentrated form before it can overcome the nutritive stimulation of a culture medium favorable to the fungus. The range of concentration from that which gives greatest growth to that which is completely inhibitive is often quite narrow.

That the toxic effect is not due to acidity or alkalinity of the medium is evident from two considerations. First, the flax wilt fungus is able to grow well throughout a wide pH range. Second, the flax media which prevented growth ranged mostly from a pH of 3.56 to 3.60, with the extreme at 4.06, while the standard check medium was 3.52 and the flax medium which allowed prompt and abundant growth ranged as high as 5.00 to 6.35 when ready for inoculation. Autoclaving the flax medium sometimes raised the pH as much as a point, although at other times only a few tenths. At two different times in the course of this work abundant growths

of foreign fungi were found in flax media which completely prevented the growth of *F. lini*. In each case it was one out of three identical flasks which had become infected. One of these was *Monilia sitophila* and the other an *Aspergillus*. This indicates that the toxic material in flax is at least somewhat specific for the *Fusarium*, but many further tests must be made to determine the range of toxic effect upon fungi in general and plant pathogenes in particular. A test was made to determine the effect of the toxic substance upon the fungus by transferring a mass of mycelium from a flax extract in which it had failed to grow into a flask of Medium A. There was no growth from this inoculum, showing that the flax medium had killed the fungus, both mycelium and spores, and not simply prevented further growth.

### ETHEREAL AND ALCOHOLIC FLAX EXTRACTS

Flax powders have been extracted with ether and with alcohol by the use of the Soxhlet apparatus and also by suction of the cold reagent through the plant material in a Buechner funnel. The following summary of such experiments will indicate the methods and the results. In Experiment 126 ✻ 4 flax powder was extracted with ether in a Soxhlet apparatus until practically no green color remained to be extracted. The powder was dried and made into a standard flax extract. Flask cultures, made as usual, were inoculated with ✻ 7 *F. lini*. Abundant growth resulted. Some of the same powder was in a similar manner extracted with alcohol and the extracted flax made into flask cultures. The fungus grew well. As a check some of the same powder was made into a culture medium without having been extracted with ether or alcohol. No fungous growth took place. Evidently the ether and alcohol treatments had removed the toxic principle, at least below the inhibitive concentration. The ether and alcohol extracts were evaporated to dryness, taken up in a small amount of hot water, filtered, and made into culture media. When inoculated with the fungus a very slow development took place in the culture from the ether extractive, showing, however, only after 12 days of incubation. No growth of *Fusarium* occurred on the culture from the alcoholic extractives, although a contamination of *Monilia sitophila* took place and grew vigorously. Evidently

at least a portion of the toxic quality was transferred from the flax by means of the ether and the alcohol and hence is soluble in these reagents. In another experiment an alcoholic extract was made by treating the powder in a Buechner funnel with successive portions of alcohol, and applying suction. The extract was then evaporated to dryness, taken up in hot water, filtered and made into flask cultures. After inoculation with the fungus and incubation for eleven days with no growth resulting, a re-inoculation was made and no growth was developed. Another ether extraction experiment was tried in which the resulting extract was concentrated and then added to a series of tubes of Medium A. The first tube has 1/10 cc., the second 2/10 cc., etc., up to 5/10 cc. Apparently no growth took place in the more concentrated tube, a slight growth in the next lower, and so on down to the least concentrated tube in which a good growth took place, although not as rapid or abundant as in the check Medium A. Exact studies of the concentration limits for these extracts have not been made. In some few cases ether extractions have failed to produce toxic cultures, but lack of proper material has made it impossible to determine the cause of these failures, although it is suspected that it is associated with the question of the source of the flax powder. Attempts to obtain a crystallized product from these ether and alcohol extracts from rather small quantities of flax have so far failed, but will be renewed when there is a sufficient supply of dry flax of known toxicity available.

## General Discussion and Conclusions

In experiments of the kind reported here there are two factors acting counter to one another. In the plant extract there are definite food values which are added to those included in the nutrients of the check medium. These would tend to increase the total growth of the fungus over the amount in the check cultures. The toxic quality must be sufficiently strong to overcome the nutritive value of the entire culture medium. In Fermi's solution used in the first studies (Reynolds, '26) the glycerine is a poor source of carbon for this fungus. Glucose, used in the check medium in the studies reported here, is a very satisfactory source of carbon. Thus while the dry weight of fungus in Fermi's

solution was usually less than 100 mgs., that in Medium A was usually from 200 to 300 mgs. The more dilute flax extracts used in the first study definitely retarded the growth of *Fusarium* in Fermi's medium, although no complete inhibition was found. In the more favorable Medium A the dilute flax extract served mainly to add nutrients and the toxic quality was thus masked. In the more concentrated flax media, from one-half to full strength, the toxic quality was evidently strong enough under some circumstances to over-balance completely the nutritive values and even kill the fungus. Since in many experiments the flax material was left long enough for the linase to hydrolyze at least a large part of the glucoside, linamarine, into HCN, acetone, and glucose, and the autoclaving produced sufficient heat to drive off the volatile toxic materials, it is clear that there must be in the flax a second toxic substance. In most of the experiments autoclaving of the flax extract did not so reduce its toxicity that the fungus could grow at the standard strength, yet occasionally at somewhat less than such a concentration the autoclaved medium did allow some growth. At about half-standard concentration autoclaving so reduced the toxicity that the added nutritive materials of the flax extract caused a distinctly larger fungous growth than in Medium A. At still lower concentrations autoclaving had little effect on the quantity of growth. It seems probable then that this second toxic material is somewhat thermostable although not completely so. Its other characteristics, as brought out in the experiments reported, are solubility in water, ether, and alcohol, and its essential non-volatility. It is somewhat specific for certain fungi and seems not to greatly influence others. While it does not dialyze readily it is not a coagulable protein. Its toxicity is rather low but when in sufficient amount it is absolutely deadly to this *Fusarium*.

Very little information appears in botanical writings concerning any such toxicity as reported here. Osterhout ('25) has reported that cells of *Valonia macrophysa* placed in sap extracted from similar cells quickly die. This result he attributed to the contrasting salt concentrations, but he did not eliminate the possibility that early death was due to other toxic factors. Prát ('27) heated extracts of several plant tissues and showed that living

cells from the same tissues die more quickly in these extracts than in isotonic sea-water of similar pH or in tap water. This seemed to indicate a special toxic action. O'Connor ('27) reported that specific, inhibiting, diffusible substances from plant and animal tissues, named by him "speciamines," inhibited growth of pollen tubes of "foreign pollens." Newton and his associates ('29) have noted an inhibiting action of wheat leaf filtrate on the germination of urediniospores and have suggested that phenolic substances are responsible for this action. It is probable that some of these observations, especially the last two mentioned, are in the same category as those reported for flax extracts.

Several materials known or supposed to exist in plants or in plant extracts should be considered in an attempt to determine the chemistry of the toxic material. Since formaldehyde may be formed from chlorophyll under certain circumstances (Warner, '14) and has been reported in the sap of green plants (Angelico and Catalano, '13), and since the flax extracts contain much leaf pigment it was thought that possibly toxicity might be ascribed to this compound. Tests were made for formaldehyde in these extracts but with entirely negative results. Mazzetti ('28) has shown that although boiled linseed oil develops bactericidal properties, these do not appear in the raw product from flax, almond, soybean, and castor bean. Since toxicity is found in unheated flax extracts, oil of the character of those named cannot be responsible for the inhibiting action. High relative acidity, coagulable proteins, and dialyzable substances have apparently been eliminated by experimental results. Toxic phenolic substances, such as suggested by Newton and his associates ('29), have not been specifically studied.

The different degrees of parasitism as shown by Broadfoot ('26) to exist in the different strains of *F. lini* should be considered in relation to resistance. In one experiment (※ 101) ※ 2 *F. lini*, which in Medium A and dilute flax cultures produced less growth than ※ 7 *F. lini*, gave a much greater growth, especially in shoot extract which has been shown to be strongly toxic. It appears that the greater virulence of ※ 2 *F. lini* is associated with its ability to resist the toxic effects of flax, rather than with any special adjustment to nutritive qualities of its host.

That resistance to flax wilt and the toxicity of flax extracts may be associated phenomena is suggested not only by the more or less specificity of these extracts for *Fusarium lini*, but also by the relation of both phenomena toward changes of environment. It has been a rather common belief among those who have worked considerably with flax that it is easily influenced by the environment. Several specific evidences are discussed by Armstrong and Eyre ('12), and in the study of varietal distribution of HCN this apparent environmental effect was noted. The degree of toxicity of flax extracts seems also to vary with the environment surrounding the growing flax as evidenced by the markedly lower toxicity of the extracts from flax grown at St. Louis as contrasted with those from flax grown in New York. Resistance to flax wilt is known to vary with temperature as noted by Tisdale ('11) and studied by Jones and others ('26) more in detail, and it is possible that other environmental factors also are important.

It is realized by the writer that a number of important suggestions made here must be much more carefully studied, but the general trend of this investigation seems to be well established and details will be studied further as time will allow.

## SUMMARY

A general discussion of some of the essential problems in the physiology of plant disease is given, with emphasis upon the need of attacking them as a special project, with the active coöperation of variously trained specialists, rather than merely as an incident in routine pathologic investigations.

By cultural studies two kinds of toxic substances are recognized in flax extracts.

The glucoside, linamarine, producing HCN upon hydrolysis, has been discussed previously. Numerous analyses for HCN in flax extracts show a great variability of amount of this glucoside: its probable presence in larger amounts in the more resistant strains of flax; and its apparent close association with the young, actively functioning cells.

A new, somewhat thermostable, toxic material appears in the flax extracts of higher concentration. This material is apparently non-dialyzable, soluble in water, ether, and in alcohol; and varies

in quantity both in relation to environmental factors and in relation to variety of flax. In many extracts it is completely inhibitive to *Fusarium lini* at the normal concentration of the flax juice.

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